Forest Pest Management
Pacific Northwest Region

FUNGICIDE TOLERANCE OF BOTRYTIS CINEREA ISOLATES FROM CONIFER SEEDLINGS

Abstract

Twenty-four *Botrytis cinerea* isolates from Pacific Northwest forest nurseries were grown on fungicide-amended media. Each isolate was tested for tolerance to seven fungicides. Overall, dichloran (Botran) was most inhibitory; variable inhibition occurred with benomyl, captan, chlorothalonil (Daconil 2787), and vinclozolin (Ronilan); and all were tolerant to some extent to mancozeb (Dithane M-45). Isolates showing tolerance to dichloran and captan generally were more tolerant to other fungicides than were isolates sensitive to dichloran and captan. Grey mold management alternatives in forest nurseries are discussed.

Introduction

Grey mold of conifer seedlings, caused by the fungus *Botrytis cinerea* (Fr.) Pers., occurs sporadically in Pacific Northwest forest bareroot nurseries, greenhouses, and storage facilities. Damage seems to be most common in greenhouses. Mortality may occur, but more frequently, a portion of the infected tree is killed and it is culled. Disease spread is enhanced by high humidity, poor air circulation, cool temperatures, high seedling density, susceptible hosts, and abundant inoculum. Spread in storage can occur if infected trees, soil, or plant debris are packed with healthy trees and if storage conditions are favorable for proliferation of *B. cinerea*.

Botrytis cinerea has a wide host range, including a number of native coniferous trees and shrubs and numerous ornamentals. Aerial spores (conidia) are produced abundantly on infected plant material and are dispersed easily by air movement or wind.

Grey mold in conifer nurseries has been controlled by regulating environmental conditions when possible, reducing seedling density, or by applications of fungicides. A number of fungicides are now available and registered for control of *B. cinerea* on a variety of crops and ornamentals. Control success in conifer nurseries has varied with different fungicides and from year to year with the same fungicide. Resistance of *B. cinerea* to the fungicide benomyl is known to exist in some conifer nurseries (Gillman and James 1980, McCain and Smith 1978).

The objectives of the following evaluation were to: (1) determine the effectiveness of several commonly used fungicides in inhibiting the growth on artificial media of *B. cinerea* isolates obtained from several conifer nurseries throughout the Pacific Northwest and (2) develop recommendations for managing grey mold in PNW nurseries.

Methods

Seedlings infected with *B. cinerea* were collected from Pacific Northwest nurseries with potential grey mold problems. A total of 26 isolates were tested, 14 from bareroot stock, 10 from containerized stock from greenhouses or shadehouses, and 2 benomyl reference isolates, (one known to be tolerant to benomyl and one sensitive to benomyl)[1/]. Isolates were from Douglas-fir *(Pseudotsuga menziesii)*, western larch *(Larix occidentalis)*, Sitka spruce *(Picea sitchensis)*, lodgepole pine *(Pinus contorta)*, and Western hemlock *(Tsuga heterophylla)* seedlings in Oregon, Washington, and Idaho nurseries.

Isolations were made on potato dextrose agar (PDA) amended with 20 ppm streptomycin sulfate. Isolates were maintained on PDA slants and refrigerated at 8°C until tested.

Fungicide tolerance of *B. cinerea* was determined by radial growth of isolates on fungicide-amended media. Amended media was prepared by adding fungicide to melted PDA to yield a concentration of 50 ppm active ingredient. Aqueous stock solutions of fungicides were prepared prior to incorporation into PDA. The appropriate amount of fungicide solution was added after media was autoclaved and cooled to 55°C. Eight mm plugs of mycelium and spores from the outer edge of 4-day-old colonies were transferred to petri plates containing 20 ml of amended PDA. Three replicate plates were made for each isolate for each of seven fungicides tested. Plates were incubated in the dark at room temperatures for 6 days after which radial growth was recorded and average growth of three replicates calculated. Trade names, manufacturers, and registration status for all fungicides are listed in Table 1.

Results

Radial growth of each isolate on fungicide-amended media, expressed as percent of growth on non-amended media (check), is given in Table 2. For each isolate, different letters following treatment means indicate significant differences (P = 0.01) based on Tukey's test for multiple comparisons of treatment means.

The fungicide, dichloran (Botran), was the most inhibitory to tested isolates; 81% (21/26) of isolates showed total, or nearly total, inhibition of growth on dichloran-amended media. Benomyl and vinclozolin (Ronilan) also were capable of causing complete growth inhibition of a number of isolates; 27% (7/26) of the isolates showed no growth on benomyl and 50% (13/26) showed no growth on vinclozolin. Generally, tolerance to benomyl was either very high or very

1/ All Idaho isolates were provided by Dr. Robert James, Pathologist, FPM, USDA, FS, Missoula, Montana. Benomyl reference isolates were provided by Dr. A.H. McCain, Extension Plant Pathologist, University of California, Berkeley.

Methods

Seedlings infected with *B. cinerea* were collected from Pacific Northwest nurseries with potential grey mold problems. A total of 26 isolates were tested; 12 from bareroot stock, 12 from containerized stock from greenhouses or shadehouses, and 2 benomyl reference isolates, (one known to be tolerant to benomyl and one sensitive to benomyl)1/. Isolates were from Douglas-fir (*Pseudotsuga menziesii*), western larch (*Larix occidentalis*), Sitka spruce (*Picea sitchensis*), lodgepole pine (*Pinus contorta*), and Western hemlock (*Tsuga heterophylla*) seedlings in Oregon, Washington, and Idaho nurseries.

Isolations were made on potato dextrose agar (PDA) amended with 50 ppm streptomycin sulfate. Isolates were maintained on PDA slants and refrigerated at 4°C until tested.

Fungicide tolerance of *B. cinerea* was determined by radial growth of isolates on fungicide-amended media. Amended media was prepared by adding fungicide to melted PDA to yield a concentration of 10 ppm active ingredient. Aqueous stock solutions of fungicides were prepared prior to incorporation into PDA. The appropriate amount of fungicide solution was added after media was autoclaved and cooled to 55°C. Eight mm plugs of mycelium and spores from the outer edge of 4-day-old colonies were transferred to petri plates containing 20 ml of amended PDA. Three replicate plates were made for each isolate for each of seven fungicides tested. Plates were incubated in the dark at room temperature for 6 days after which radial growth was recorded and average growth of three replications calculated. Trade names, manufacturers, and registration status for all fungicides are listed in Table 1.

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Radial growth of each isolate on fungicide-amended media, expressed as percent of growth on non-amended media (check), is given in Table 2. For each isolate, different letters following treatment means indicate significant differences ($p = 0.01$) based on Tukey's test for multiple comparisons of treatment means.

The fungicide, dichloran (Botran), was the most inhibitory to tested isolates; All 26 (100%) of isolates showed total, or nearly total, inhibition of growth on dichloran-amended media. Benomyl and vinclozolin (Ronilan) also were capable of causing complete growth inhibition of a number of isolates; 27% (7/26) of the isolates showed no growth on benomyl and 50% (13/26) showed no growth on vinclozolin. Generally, tolerance to benomyl was either very high or very

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TABLE 1. Fungicides incorporated into media to test tolerance of *Botrytis cinerea* isolates.

Product name	Common name	Manufacturer	Registration on Conifers for *Botrytis*
Benlate 50 WP	benomyl	Dupont	yes-O,W,I[1/]
Benomyl	benomyl	Lilly-Miller	no
Botran 75 W	dichloran	Tuco	yes-O,W
Captan 50 W	captan	Millers	no[2/]
Daconil 2787	chlorothalonil	Diamond-Shamrock	yes-O,W
Dithane M-45	mancozeb	Rohm & Haas	no[3/]
Ronilan 50 W	vinclozolin	BASF	no[4/]

[1/] O = Oregon, W = Washington, I = Idaho

[2/] Registered for use on shrubs and trees for damping-off and root rot.

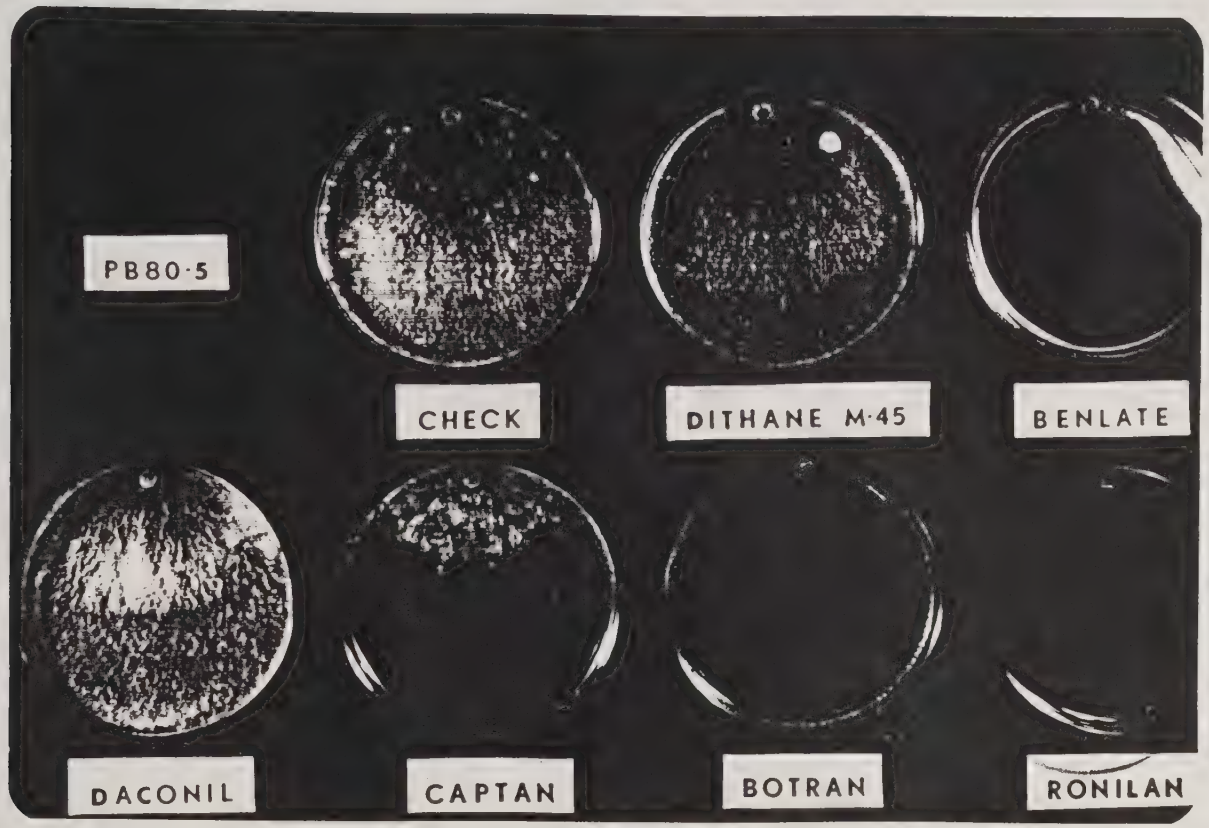
[3/] Registered for use on conifers for foliage diseases caused by Lophodermium fungi.

[4/] Has experimental use permit on strawberries for *Botrytis* control.

low, with few intermediate responses (Figure 1). All isolates showed similar responses to both benomyl products. All isolates were tolerant, to some extent, to mancozeb (Dithane M-45) where the range of radial growth was between 39% and 100% of the check. Variable tolerance was found to captan and chlorothalonil (Daconil 2787).

Tolerance to more than one fungicide was frequent for individual isolates. Cross-tolerances, in some cases, appear to exist. As shown in Tables 3-5, isolates tolerant to dichloran show greater tolerance to other fungicides and those isolates sensitive to dichloran also are sensitive to a greater number of other fungicides. Similarly, isolates sensitive to captan are more sensitive to benomyl, vinclozolin, and chlorothalonil than are captan-tolerant isolates. No cross tolerance is apparent with benomyl.

Figure 1. Growth of Isolates PB80-5 and PB80-6 on fungicide-amended PDA after 14 days. Differential responses to benomyl are obvious.



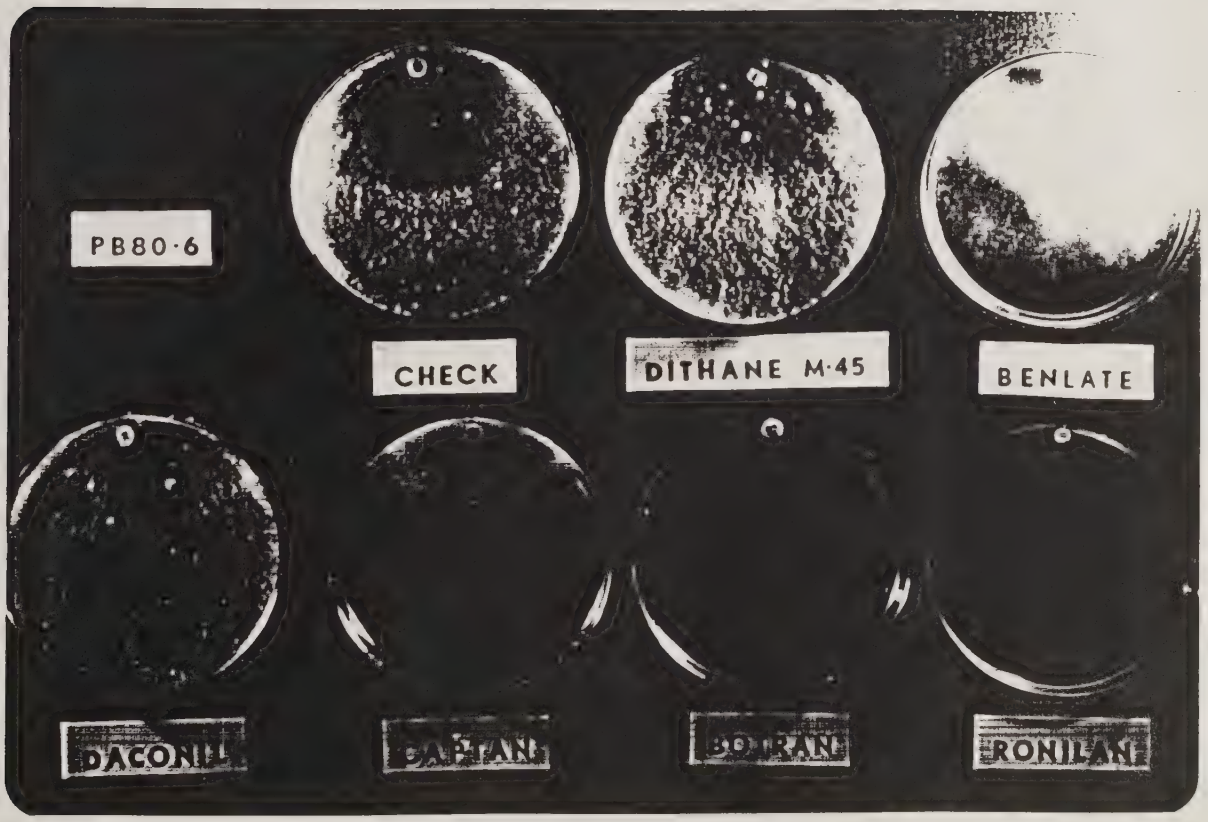


TABLE 2. Effect of fungicides on growth of *Botrytis cinerea*[1/]

CONTAINER STOCK

Fungicide Treatment	CALIFORNIA[2/]		OREGON					WASHINGTON				IDAHO
	Benomyl sensitive	Benomyl tolerant	FG 80-1 DF[3/]	FG 80-2 DF	FG 80-3 WL	FG 80-4 LPP	PG 80-1 WL	SG 80-1 DF	SG 80-2 SS	SG 80-3 SS	SG 80-4 SS	FG 80-6 WL
control	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A
benomyl (Dupont)	0D	51B	0D	90AB	0E	0D	74AB	76BC	56C	75ABC	78C	74B
benomyl (Lilly-Miller)	0D	50B	---	79BC	3E	0D	77AB	74BCD	52C	65BC	75C	72B
dichloran	5CD	0C	4C	32D	0E	0D	0D	31E	0E	9D	0F	0D
captan	22BC	4C	35B	93A	44D	18C	17CD	67CD	20D	72BC	19E	31C
chlorothalonil	39B	48B	29BC	79BC	52C	43B	46BC	83B	46C	78ABC	43D	44C
*mancozeb	87A	99A	90A	99A	89B	91A	94A	100A	71B	90AB	92B	---
vinclozolin	0D	0C	4C	68C	0E	0D	16CD	65D	0E	61C	0F	0D

1/Radial growth measured after 6 days growth on PDA amended with 50 ppm fungicide. Growth expressed as percent of control. Each percent represents the mean of 3 trials. Means followed by different letters indicate significant differences at P = 0.01 level using Tukey's test of multiple comparisons.

2/Reference isolates known to be tolerant or sensitive to benomyl. Provided by A.H. McCain, U.C., Berkeley.

3/DF = Douglas-fir; WL = western larch; LPP = lodgepole pine; SS = Sitka spruce; WH = western hemlock.

TABLE 2. continued:

BAREROOT STOCK

Fungicide Treatment	OREGON			WASHINGTON									IDAHO	
	PB 80-1 DF	PB 80-2 SS	PB 80-3 DF	PB 80-4 DF	PB 80-5 DF	PB 80-6 DF	PB 80-7 DF	PB 80-8 DF	PB 80-9 DF	PB 80-10 WH	FB 80-1 DF	FB 80-2 WL	FB 80-5 LPP	FB 80-6 WL
control	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A	100A
benomyl (Dupont)	71D	78B	72BC	69AB	0C	75B	72A	72CD	71B	65B	0D	0D	75A	79B
benomyl (Lilly-Miller)	74CD	79B	84B	77AB	0C	70B	79A	79BC	77B	74B	0D	0D	72AB	79B
dichloran	2E	0E	25E	53B	0C	0D	0B	0E	0D	0D	0D	0D	0D	0D
captan	86B	15D	47D	47B	19BC	21C	9B	92A	9C	9D	31C	42C	38BC	19C
chlorothalonil	78C	43C	71BC	48B	38B	29C	22B	82B	21C	22C	35BC	49C	35CD	22C
mancozeb	---	---	---	---	---	---	---	---	---	---	39B	85B	---	---
vinclozolin	72CD	0E	63C	59B	9BC	1D	11B	68D	7D	0D	0D	0D	0D	0D

TABLE 3: Growth of dichloran-tolerant and dichloran-sensitive isolates on PDA amended with other fungicides.

	DICHLORAN-TOLERANT ISOLATES						
	PB 80-4	FG 80-2	SG 80-1	PB 80-3	SG 80-3	FG 80-1	PB 80-1
	53[1]	32	31	25	9	4	2
benomyl[2]	+++[3]	++++	++++	+++	++++	0	+++
captan	++	++++	+++	++	+++	++	++++
chlorothalonil	++	+++	++++	+++	++++	++	++++
vinclozolin	+++	+++	+++	+++	+++	+	+++

DICHLORAN-SENSITIVE ISOLATES						
PB 80-2	PB 80-6	PB 80-9	FB 80-1	FG 80-3	PG 80-1	SG 80-4
0	0	0	0	0	0	0
++++	++++	+++	0	0	+++	++++
+	+	+	++	++	+	+
++	++	+	++	+++	++	++
0	+	+	0	0	+	0

1/Radial growth on PDA + 50 ppm fungicide after 6 days, expressed as percent of control.
2/Benlate 50WP, Dupont.
3/++++ = 76 - 100% of control, +++ = 51 - 75%, ++ = 26 - 50%, + = 1 - 25%, 0 = 0 growth.

TABLE 4: Growth of captan-tolerant and captan-sensitive isolates on PDA amended with other fungicides.

	CAPTAN-TOLERANT ISOLATES[4]				
	FG 80-2	PB 80-8	PB 80-1	SG 80-3	SG 80-1
	93[1]	92	86	72	67
benomyl[2]	++++[3]	+++	+++	+++	++++
dichloran	++	0	+	+	++
chlorothalonil	++++	++++	++++	++++	++++
vinclozolin	+++	+++	+++	+++	+++

CAPTAN-SENSITIVE ISOLATES[5]				
FB 80-6	FG 80-4	PB 80-1	PB 80-2	PB 80-7
19	18	17	15	9
++++	0	+++	++++	+++
0	0	0	0	0
+	++	++	++	+
0	0	+	0	+

1/Radial growth on PDA + 50 ppm fungicide after 6 days, expressed as percent of control.
2/Benlate 50WP, Dupont.
3/++++ = 76 - 100% of control, +++ = 51 - 75%, ++ = 26 - 50%, + = 1 - 25%, 0 = 0 growth.
4/Isolates showing growth > 50% of control.
5/Isolates showing growth < 20% of control.

TABLE 5: Growth of benomyl-tolerant and benomyl-sensitive isolates on PDA amended with other fungicides.

	BENOMYL[2/]-TOLERANT ISOLATES						BENOMYL-SENSITIVE ISOLATES					
	FG 80-2	SG 80-4	SG 80-1.	PB 80-6	PB 80-1.	PB 80-10	PB 80-5	FG 80-1.	FG 80-2	FG 80-1	FG 80-3	FG 80-4
	90[1/]	78	76	75	71	65	0	0	0	0	0	0
dichloran	++[3/]	0	++	0	+	0	0	0	0	+	0	0
captan	++++	+	+++	+	++++	+	+	++	++	++	++	+
chlorothalonil	++++	++	++++	++	++++	+	++	++	++	++	+++	++
vinclozolin	+++	0	+++	+	+++	0	+	0	0	+	0	0

1/ Radial growth on PDA + 50 ppm fungicide after 6 days, expressed as percent of control.
2/ Benlate 50WP, Dupont.
3/ ++++ = 76 - 100% of control, +++ = 51 - 75%, ++ = 26 - 50%, + = 1 - 25%, 0 = 0 growth.

Discussion

Exclusive use of one fungicide can result in the development of local resistant populations of *B. cinerea* (Dekker 1976, Ogawa et al 1976). Resistant individuals, already present in the population at very low frequencies or arising through mutations, are not eradicated and can reproduce without competition. Continued use of one fungicide allows this resistant population to become dominant so that fungicide applications are no longer effective. There are a number of fungicides, including captan, ferbam, dichloran, and benomyl, to which certain local populations of *B. cinerea* have developed resistance (Bollen and Scholten 1971).

Our results show that a number of the tested isolates are resistant to benomyl as well as to captan and vinclozolin at concentrations of 50 ppm. It has been assumed that resistance to benomyl and captan has been acquired over time through selection of resistant populations by continued nursery use of these fungicides. However, Gillman and James (1980) found tolerance to benomyl in two nurseries where it had not been used previously. They suggest that tolerance either developed very rapidly or that resident populations were already tolerant to benomyl. Similarly, tolerance exists to vinclozolin, which has not been used in forest nurseries. Tolerance of vinclozolin appears to be widely present in the resident natural *B. cinerea* populations. Presumably, exclusive use of vinclozolin would lead to very rapid build-up of tolerant populations due to presence of the already high proportion of vinclozolin-resistant individuals in the population.

Extreme sensitivity to dichloran was found in all but four isolates (FG 80-2, SG 80-1, PP 80-3, PB 80-4), all from different locations. Tolerance of dichloran by *B. cinerea* in nurseries has not been reported previously (Gillman and James, 1980) although it has been reported in laboratory studies by Webster et al (1970).

Webster and others (1920) suggest that low levels of tolerance exist in a population of *B. cinerea* and can become dominant when selection pressure is exerted by use of a chemical. They also suggest that varying levels of tolerance may be due to a heterocaryotic state (more than one nucleus per cell) where each nucleus may contain a tolerant factor. Heterocaryosis may explain the intermediate levels of tolerance seen in this study to other fungicides such as captan and chloro- thalonil.

Tolerance to both captan and chlorothalonil was relatively high, despite reports of effectiveness elsewhere (Gillman and James, 1980; McCain and Smith 1978; Ogawa et al 1976).

Cross-tolerance was apparent between several fungicides. Miller and Fletcher (1974) found that cross-tolerance occurs between related benomyl products, where *B. cinerea* isolates tolerant to benomyl also were tolerant to the related compounds thiophanate-methyl, thiabendazole, and BAS 3460F.

Forest nurseries cannot rely on any one fungicidal product for control of grey mold for extended periods of time due to potential rapid acquisition or currently present fungicide tolerance in populations of *B. cinerea*. Tolerance of even new products may develop very rapidly or may be present already in *Botrytis* populations. Chemical suppression of grey mold must, therefore, depend on a number of products with varying degrees of effectiveness, each aimed at different segments of the *Botrytis* population.

Management alternatives for control of grey mold are given in order of least to most effective in reducing disease losses caused by *Botrytis cinerea*.

1. Use of one currently effective fungicide. Good disease suppression may be achieved for a period of time. Tolerance would be expected to arise at some point in the future followed by decreased effectiveness of the fungicide and increased crop losses.

2. Use of several different fungicides applied in rotation. Adequate disease suppression would be expected for an extended period of time. It is advisable to use the lowest concentration of fungicide that will adequately control the disease and, if possible, select fungicides with different modes of action against *B. cinerea*.

3. Use of good cultural practices. Reduced stocking density, good air circulation among plants, and less frequent irrigation (to reduce moisture retention on foliage) are several means of creating a less favorable environment for spread and infection by *B. cinerea*. Inoculum loads can be reduced by good greenhouse sanitation and removal and destruction of plant debris and infected plants. Control success will vary, to some extent, with climatic fluctuations.

4. Combine good cultural practices with use of several different fungicides applied in rotation. Optimal disease suppression can be expected when good cultural modifications and practices (as described in management alternative 3) are coupled with fungicide applications. Periodic tests of *Botrytis* isolates from each nursery are desirable to determine if tolerance to fungicides in use has developed.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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Literature Cited

Bollen, G.J. and G. Scholten. 1971.
Acquired resistance to benomyl and some other systemic fungicides in a strain of *Botrytis cinerea* in cyclamen. Netherland Journal of Plant Pathology. 77:83-90.

Dekker, J. 1976.
Acquired resistance to fungicides. Annual review of phytopathology. 14:405-428.

Gillman, L. and R.L. James. 1980.
Fungicidal tolerance of Botrytis within Colorado greenhouses. Tree Planters' Notes. Winter 1980. pg. 25-28.

McCain, A.H. and P.C. Smith. 1978.
Evaluation of fungicides for control of Botrytis blight. Tree Planters Notes. 29(4):12-13.

Miller, M.W. and J.T. Fletcher. 1974.
Benomyl tolerance in *Botrytis cinerea* isolates from glasshouse crops. Transactions, British Mycological Society. 62(1):99-103.

Ogawa, J.M., B.T. Manjii, and G.A. Chastagner. 1976.
Field problems due to chemical tolerance of plant pathogens. Proceedings, American Phytological Society. Soc. 3:47-53.

Webster, R.K., J.M. Ogawa, and E. Bose. 1970.
Tolerances of *Botrytis cinerea* to 2,6-dichlor-4-nitroaniline. Phytopathology. 60:1,489-1,492.

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